

source: 119 PB/31 BM) were retrospectively analyzed. Their diagnoses were 90 acute leukemia, 30 CML, 6 MDS and 24 other diseases. Multiplex PCR method was performed to amplify 16 STR loci (D3S1358, HUMvWA, D16S539, D2S1338, Amelogenin, D8S1179, D21S11, D18S51, D19S433, THO1, FGA, D7S820, CSF1PO, D13S317, TPOX, D5S818) (ABI Prism 3130). The loci examined were classified as complete matched (CM), partially matched (PM), and fully mismatched (FMM) between donors and recipients.

**Results:** The loci of D13S317, D18S51 and D2S1338 were the most informative, while the loci of TPOX and CSF1PO were the least. The incidence of acute GvHD was 46.7% (n = 69), which acute severe GvHD (grII-IV) was observed in only 31 patients. Chronic GvHD was developed in 63.4% patients. The incidence of grII-IV GvHD was higher in patient with CM in TPOX loci (p = 0.02). Chronic GvHD was more frequent in the patients with PM in D5S818 loci than those with CM or MM (p = 0.016). While PM D21S11 increased TRM, MM or PM in D5S818 loci decreased the TRM. In our cohort analysis, 2-year probability of disease-free survival(DFS) and overall survival(OS) were  $58.1 \pm 5.5\%$  and  $67.5 \pm 4.4\%$ , respectively. The CM in D21D11 locus (p = 0.07) and PM in D5S818 locus (p = 0.009) prolonged the probability of DFS. In multivariate analysis, these loci had an impact on DFS (p = 0.055 vs p = 0.005). D19S433 and D5S818 loci had an effect on the OS. We repeated similar analyses into two groups, mismatched (MM) group, which FMM and PM was accepted as a whole group or CM group, while the incidence of grII-IV GvHD was higher in patients with CM of D18S51 and TPOX loci, the chronic GvHD was more frequent in those with CM D5S820 loci. Similar as the first analyses MM in D21S11 and CM in D5S818 affected both TRM and DFS. Besides, MM in FGA locus decreased TRM and prolonged DFS. The impact of D5S818 on the OS also continued, additionally D19S433 had minimal effect on the OS. In conclusion, some disparities of STR loci might affect the transplantation outcome; however, these results should be analyzed together with other co-variables and on multicenter basis.

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#### AN EVALUATION OF DNA METHYLATION CHANGES IN A PHASE I CLINICAL TRIAL OF LOW-DOSE 5-AZACITIDINE (AZA) GIVEN AS MAINTENANCE THERAPY AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

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DNA methylation of CpG Islands is a frequently found epigenetic alteration in AML and MDS that leads to deranged gene activity. Inhibition of the enzyme DNA methyltransferase is associated with hypomethylation, and possibly, restoration of normal function to genes critical for differentiation and proliferation. AZA is a DNA hypomethylating agent that may induce increased tumor immunogenicity, potentially magnifying the GVL effect. Lower doses are likely to be better tolerated after HSCT and to be effective inducers of hypomethylation. We hypothesized that AZA after HSCT will lower relapse rates, and designed a phase I clinical trial that also uses a molecular surrogate endpoint for dose finding. Here we describe methylation changes in patients so treated.

Pts with AML or high-risk MDS not in first remission (CR) are eligible. Three doses of AZA were studied: 8, 16, and 24 mg/m<sup>2</sup> daily  $\times$  5 starting on day + 42. DNA was extracted from mononuclear cells of 22 pts that received AZA. The methylation status of long interspersed nuclear elements (LINE) was analyzed by pyrosequencing as a surrogate marker of global DNA methylation before and after AZA administration. Gene specific methylation changes were studied using a methylated CpG island amplification(MCA)/CpG array.

Median age was 57 years. Diagnoses were MDS (n = 4) and AML(n = 18). Disease status at HSCT: CR, 18% (n = 4), and active disease, 82%(n = 18). LINE methylation results were as follows: baseline: 44.51%; on cycle 1, 5th day of AZA: 24 mg/m<sup>2</sup> = 43.58%; 16 mg/m<sup>2</sup>: 36.93%; 8 mg/m<sup>2</sup>: 42.86%. Two pts have re-

lapsed while on AZA. There has been no major drug-related toxicity and no increase in GVHD incidence. Analysis of gene-specific methylation is ongoing.

AZA in doses up to 24 mg/m<sup>2</sup> can be safely administered early after HSCT. Given the lack of toxicity and low levels of changes in DNA methylation observed, we are currently investigating higher doses.

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#### ROLE OF EXTENSIVE SPLENOMEGALY IN PATIENTS WITH MYELOFIBROSIS UNDERGOING A REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANT

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Allogeneic stem cell transplantation has been shown to restore normal hematopoiesis in patients with intermediate or high risk primary myelofibrosis (PMF) or myelofibrosis preceded by polycythemia vera or essential thrombocythemia. However, in patients with PMF and extensive splenomegaly it is not clear whether transplant is associated with an unacceptable risk or if splenectomy should be performed prior to transplant. In this study, ten consecutive patients with myelofibrosis who were not splenectomized received an allogeneic hematopoietic stem cell transplantation (HSCT) from related or unrelated donors and were periodically monitored by ultrasound or CT scan for 12 months after transplant to assess the kinetics of the reduction of splenomegaly. These findings were correlated with the time to resolution of marrow fibrosis, time to engraftment and clinical outcome. Over a 12 month period a progressive reduction in spleen size was observed in all the patients and paralleled the reduction in marrow fibrosis. Of 10 patients, 5 with a splenic longitudinal diameter  $>30$  cm showed a more prolonged time to engraftment ANC  $> 0.5 \times 10^9/L$  (d 19  $\pm$  5 vs 13  $\pm$  2, p = 0.05) and platelet  $> 20 \times 10^9/L$  (d 75  $\pm$  104 vs 11  $\pm$  2, p = 0.06). However, of the 5 patients with more extensive splenomegaly only 2 experienced delayed engraftment of platelets (d 77 and 256, respectively). Full donor chimerism was observed in all patients within 60 days after transplantation regardless of the size of the spleen. At a median follow-up of 51 months (range: 2-81) all the patients are alive but 1 who died of a TTP like syndrome 2 months after transplant and had a small spleen at the time of transplant. A clinical CR has been documented in 7 patients and 2 have achieved clinical improvement according to the criteria established by the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT). These findings suggest that allogeneic HSCT with RIC regimen can be safely performed in patients with extensive splenomegaly. Although in these patients the time to achieve a full hematopoietic engraftment may be prolonged, the significant risk of morbidity and mortality associated with splenectomy may be avoided.

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#### EFFECT OF SUBSTITUTING FLUDARABINE AND THYMOGLOBULIN FOR CYCLOPHOSPHAMIDE IN BUSULFAN-BASED CONDITIONING REGIMENS ON T-CELL CHIMERISM AND OUTCOMES AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION (HCT)

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Substitution of cyclophosphamide by agents such as fludarabine and thymoglobulin in busulfan-based conditioning regimens has been used to reduce complications after allogeneic HCT. We performed a retrospective analysis of outcomes among 348 patients

who had HCT since 2001 with the use of 5 myeloablative conditioning regimens: BuCY  $\pm$  Thymoglobulin, FluBu, or FluBu + Thymoglobulin with different doses of Flu designated Flu[1(120 mg/m<sup>2</sup>)]BuThy and Flu[2(250 mg/m<sup>2</sup>)]BuThy. Grafts consisted of unmanipulated bone marrow or growth factor-mobilized blood cells from related or unrelated donors, except for 9 patients who received BuCY and mobilized blood cells from related donors that were 90% depleted of T-cells. Donor chimerism in recipient blood T-cells (CD3) and granulocytes (CD33+) was measured at day 80 after HCT.

Patient characteristics in the 5 groups were similar: median age 49, 91% MDS or AML (64% high-risk), 51% unrelated donors, 88% PBSC, 59% HCT-CI  $\geq 1$ . Donor chimerism of granulocytes was complete by day +80 in all groups, but persistent mixed T-cell chimerism was observed in certain groups. Compared to patients who received BuCY with unmanipulated allografts, the incidence of mixed chimerism with >50% recipient T-cells was higher among patients who received Flu, bone marrow grafts or T-cell depleted grafts (e.g., Flu[1]BuThy: OR 14.5,  $p < 0.0001$ ). Most patients with mixed chimerism at day 80 had full donor T-cell chimerism at 1 yr after HCT, but some patients with malignancy in complete remission at 3 yr continue to have persistent mixed T-cell chimerism.

Compared to BuCY controls, improved outcomes with reduced regimen-related toxicity were observed in the 22 patients who received Flu[1]BuThy. After adjustment for age, disease risk, donor type, HCT-CI and prior CMV infection in the recipient, the Flu[1]-BuThy group had significantly lower rates of acute GvHD (HR 0.50,  $p = 0.02$ ), chronic GvHD (HR 0.31,  $p = 0.003$ ) and NRM (HR 0.25,  $p = 0.05$ ), although relapse was not significantly increased (HR 1.13,  $p = 0.87$ ) and overall survival was not significantly improved (HR = 0.60,  $p = 0.19$ ). In contrast, NRM and survival in the Flu[2]BuThy and BuCY groups showed no statistically significant differences. In general, addition of thymoglobulin for GvHD prophylaxis significantly reduced the incidence of chronic GvHD. A prospective, randomized trial is needed to determine whether superior outcomes can be achieved after conditioning with Flu[1]BuThy as compared with BuCy.

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#### THYMOGLOBULIN® RABBIT ANTI-THYMOCYTE GLOBULINS (r-ATG) INDUCES FASTER PLATELET RECOVERY AND DOES NOT SUPPRESS GRANULOCYTE ENGRAFTMENT IN A NON-MYELOABLATIVE STEM CELL TRANSPLANTATION SETTING

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**Introduction:** The role of anti thymocyte globulin in GVHD prevention is well established, but it is also known that anti thymocyte globulin induces leucopenia and thrombocytopenia and as such might theoretically delay engraftment. Additionally, fast platelet engraftment is a marker for good hematopoietic stem cell graft function. We have prospectively compared the effect of Thymoglobulin on engraftment of granulocytes and platelets following a single non-myeoablative conditioning protocol in a randomized study.

**Methods:** 28 consecutive patients undergoing SCT were conditioned with the NST protocol (I.V. fludarabine 30 mg/m<sup>2</sup>/day (days -10 to -5) and I.V. busulfex (3.2 mg/kg on days 6 and 5), with or without I.V. Thymoglobulin according to randomization (days 4 to 1). Thymoglobulin dosing schedule: a cumulative dose of 7.5 mg/kg r-ATG was given with the following program 0.5 mg/kg day -4, 2.0 mg/kg day -3, 2.5 mg/kg day -2, 2.5 mg/kg day -1. Patients were followed for engraftment of leukocytes and platelets. **Results:** only one patient rejected the graft (conditioned without Thymoglobulin, NS). In 4 patients underlying disease progression prevented engraftment of either ANC or platelets (2 per group). The median time to ANC engraftment was similar between the groups, 15  $\pm$  2.8 and 14  $\pm$  2.9 days for the Thymoglobulin and control groups respectively ( $p = 0.21$ ). However, the median time for platelet engraftment was significantly shorter in patients receiving Thymoglobulin as part of the conditioning. Platelet recovery occurred 9.5  $\pm$  1.2 and 11  $\pm$  2.5 days for the Thymoglobulin

and control groups respectively ( $p = 0.018$ ). **Conclusions:** In the non-myeoablative setting, Thymoglobulin induces faster platelet recovery and does not delay ANC engraftment.

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#### A COMPARISON OF MEASURED VS. CALCULATED CREATININE CLEARANCE AND THEIR LACK OF ASSOCIATION WITH RENAL REGIMEN RELATED TOXICITY AFTER AUTOLOGOUS AND ALLOGENEIC BMT

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Standard BMT eligibility criteria generally include glomerular filtration rate (GFR)  $\geq 50$  ml/min for myeloablative and GFR  $\geq 40$  ml/min for nonmyeloablative regimens. The most accurate, but costly, method of GFR measurement is urinary clearance of molecular isotopes such as <sup>99m</sup>Tc-DTPA. In BMT pts, GFR is commonly estimated by creatinine clearance in a 24 hour urine collection (24 hr CrCl), a time-consuming and error-prone method. 4 equations incorporate different clinical variables to estimate CrCl: Cockcroft-Gault (CG), modified CG (mCG), Modification of Diet in Renal Disease 1 (MDRD1) and MDRD2. The study objectives were to determine which of 4 calculations consistently underestimates measured CrCl pre-BMT and to determine if any were correlated with renal regimen related toxicity (RRT). We retrospectively analyzed 100 consecutive allo ( $n = 50$ ) or auto ( $n = 50$ ) BMT pts from 1/06 to 4/07 to compare measured CrCl with each of the 4 formulas using linear regression and Wilcoxon signed rank test. Renal RRT was defined using the Bearman scale: no increase from baseline serum Cr (gr 0), any increase over baseline (gr 1), doubling of baseline (gr 2), dialysis (gr 3), or death from renal RRT (gr 4), with an incidence of 24%, 57%, 18%, 1% and 0%, respectively. All 4 formulas were significantly ( $p < .001$ ) correlated to measured CrCl with correlation coefficients (R) = .63 (CG), .61 (mCG), .50 (MDRD1) and .54 (MDRD2). When stratified by gender, CG had the highest R for women ( $R = .679$ ,  $p < .001$ ), while MDRD1 had the highest for men ( $R = .40$ ,  $p = .003$ ). MDRD1 consistently underestimated CrCl in the highest proportion of pts (65%,  $p < .001$ ), compared to MDRD2 (65%,  $p = .001$ ), mCG (54%,  $p = NS$ ) and CG (33%,  $p = .001$ ). Measured CrCl, serum Cr, and all 4 calculations were not predictive of renal RRT post-BMT. Significant univariate predictors of gr 2-3 renal RRT were: alloBMT, reduced intensity/nonmyeloablative regimens (RICNMA), KPS  $\leq 80$  and albumin  $\leq 3.7$  g/dL. In alloBMT, BMI  $> 35$ , KPS  $\leq 80$  and albumin  $\leq 3.8$  g/dL were significantly associated with gr 2-3 renal RRT, however in autoBMT baseline serum Cr  $\geq 1.1$  was the only significant factor. Any of the 4 equations can replace the 24 hr CrCl. CG is closest to, but overestimates CrCl in 67% of pts. In comparison, MDRD1 consistently underestimates CrCl in 65%. Larger analyses may better define subgroups at risk for renal RRT. Our results suggest a lower CrCl threshold may be acceptable for BMT due to the relatively low gr 2-4 renal RRT incidence observed.

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#### MYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION IN 424 PATIENTS USING BUSULFAN AND CYCLOPHOSPHAMIDE

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**Background/Aims:** From 1985 to 2007, 424 adult patients received Busulfan Cyclophosphamide (Bu<sub>4</sub>Cy<sub>2</sub>) as conditioning regimen before allogeneic stem cell transplantation in our institution. In this study we analyzed this group with regard to overall survival, LFS, TRM and the incidence of complications.

**Patient Material/Methods:** 424 patients underwent ASCT; 43% female ( $N = 185$ ) and 57% male ( $N = 239$ ). Median age was 39.9 y (15.4–60.7). 263 patients (62%) received transplants from family donors and 161 (38%) from matched unrelated donors